Fisheries and Oceans Canada | Pêches et Océans Canada

Canadian Stock Assessment Secretariat
Research Document 99/134

Not to be cited without
permission of the authors[1]

Secrétariat canadien pour l'évaluation des stocks
Document de recherche 99/134

Ne pas citer sans
autorisation des auteurs[1]

Genetic variation among populations of bowhead whales summering in Canadian waters.

Lianne D. Maiers, Brigitte G.E. de March, James W. Clayton, Larry P. Dueck and Susan E. Cosens

Fisheries and Oceans Canada
Central and Arctic Region
501 University Cr.
Winnipeg, Manitoba
R3T 2N6

[1] This series documents the scientific basis for the evaluation of fisheries resources in Canada. As such, it addresses the issues of the day in the time frames required and the documents it contains are not intended as definitive statements on the subjects addressed but rather as progress reports on ongoing investigations.

Research documents are produced in the official language in which they are provided to the Secretariat.

[1] La présente série documente les bases scientifiques des évaluations des ressources halieutiques du Canada. Elle traite des problèmes courants selon les échéanciers dictés. Les documents qu'elle contient ne doivent pas être considérés comme des énoncés définitifs sur les sujets traités, mais plutôt comme des rapports d'étape sur les études en cours.

Les documents de recherche sont publiés dans la langue officielle utilisée dans le manuscrit envoyé au secrétariat.

ISSN 1480-4883
Ottawa, 1999

Canada

GENETIC VARIATION AMONG POPULATIONS OF BOWHEAD WHALES SUMMERING IN CANADIAN WATERS

ᐊᖏᕐᕋᓱᐊᕐᓂᖅ ᐃᓕᓴᕐᕕᓂᓕᕆᓂᖅ ᐊᑦᓯᓴᕐᕕᓂᖅ ᐊᐅᓚᑦᑕᓪᓗᖅ ᑲᓇᑕᐅᑦ ᑕᓪᓕᐊᓕᓂᓂ

L.D. Maiers, B.G.E. de March, J.W. Clayton, L.P. Dueck and S.E. Cosens

ABSTRACT

Renewed aboriginal subsistence harvests of bowhead whales (*Balaena mysticetus*) in the eastern Canadian Arctic has prompted a need to more closely monitor the status of these animals. Information from the study of genetic markers can help answer questions about stock structure, distribution and movement, breeding strategies and population size dynamics. Bowhead samples from 64 individuals representing 3 putative stocks were analyzed for variability at 13 nuclear DNA microsatellite loci and along 343 base pairs (bp) of mitochondrial DNA sequence. Analyses of the data support the idea that the Davis Strait stock is distinct from the Hudson Bay/Foxe Basin and Bering Sea stocks. Results also suggest that the Hudson Bay stock is more similar to the Bering Sea stock than it is to the Davis Strait stock. This pattern could result from post-glacial colonization of the eastern Arctic by two separate and genetically distinct groups of bowheads.

Résumé

La reprise de la récolte de subsistance de la baleine boréale (*Balaena mysticetus*) par les autochtones dans l'est de l'Arctique canadien a fait naître le besoin de surveiller l'évolution du statut de cet animal. Les renseignements obtenus par l'étude de marqueurs génétiques peuvent aider à fournir des réponses à certaines questions sur la structure des stocks, l'aire de répartition et les déplacements, ainsi que sur les stratégies de reproduction et la dynamique de l'effectif de la population. Des échantillons prélevés sur 64 baleines boréales représentant 3 stocks présumés ont été analysés afin d'étudier la variabilité à 13 loci de microsatellites d'ADN nucléaire et le long d'une séquence d'ADN mitochondrial de 343 pb. L'analyse des données corroborent l'hypothèse selon laquelle le stock du détroit de Davis diffère de celui de la baie d'Hudson du bassin Foxe et de celui de la mer de Béring. De plus, ils suggèrent que le stock de la baie d'Hudson s'apparente davantage à celui de la mer de Béring qu'à celui du détroit de Davis. Cette tendance pourrait résulter d'une colonisation postglacaire de l'est de l'Arctique par des baleines boréales provenant de deux groupes distincts sur le plan de la génétique.

ᓄᒋᓗᐊᖅᑕᐅᓯᒪᔪᖅ ᓄᓇᖃᖅᖃᖏᑐᓚᓂᒃ ᓂᕆᖃᕆᓯᐊᖅᓂᒍᒃ ᐅᒪᔪᖅᑕᐅᔪᓇᖅᓱᓂᓄᖅᓄᓂᒃ ᐊᖏᕐᓂ
(Balaena Mysticetus) ᑲᓇᑕᐅᑦ ᐅᑭᐅᖅᑕᖏᑐᒃ ᐅᐊᖅᓇᖏᓂ ᐱᒋᐊᖅᑎᑎᓯᒪᔪᒃᑐᑦ
ᐱᓵᓗᐊᖃᓕᕐᒥᒃ ᖃᐅᔨᓴᖅᑕᐅᑎᑦᓭᓪᓗᑎᒃ ᑭᓯᐊ ᓂᕐᔪᐊᑎᐅᔪᓂ. ᑐᓴᒪᓴᓪᓕᐊᔾᔨᓯᒪᔪᓂ
ᖃᐅᔨᓴᖅᑕᐅᓂᖅᑎᑐᒍᑦ ᐃᓕᓴᕐᓂᓂᖅᑎᑐᒍᑦ ᐊᓯᔾᔨᓂᓂᓂᑦ ᓇᓗᓇᐃᔾᔨᑦᓂᖅᓂᒃ ᐃᖃᓗᑕᐅᔪᓐᓇᖅᑐᒃ
ᑭᐅᔪᓇᓐᓂᓗᓂ ᑲᑎᖅᖏᓪᓕᓴᕐᓂ, ᐊᖏᔪᑐᖃᓕᓂᖅᓂᒃ ᐊᖏᓗ ᓄᑦᓂᓂᖅᓂᒃ,
ᐃᓂᐅᔨᓂᓪᓗᑦ ᐊᑐᖅᑕᐅᔪᑎᑦ ᐊᖏᓗ ᐃᓄᒃᑎᑐᒍᑦ ᐊᖓᓂᖅᓂᓄᖅ ᐊᖃᖅᖏᓪᓕᓴᕐᓂᑦ.
ᓇᓕᓐᓄᓕᐊᑎᖃᕐᕕᑦ ᐃᓕᓴᕐᓂᓂᖅᑎᑐᒍᑦ ᐊᓯᔾᔨᓂᓂᓂᖅᓂᑦ ᓇᓗᓇᐃᖃᓕᓴᕐᓂᑦ ᒪᑉᑕᖅᓂᓂᖅ
ᖃᐅᔨᓴᖅᑕᐅᓂᓄᑦ 64-ᓂᑦ ᐊᖏᔪᓂᑦ ᑕᑯᓂᖅᓂᑦ ᐱᖓᓱᐃᓂᑦ ᐃᓕᓴᕐᑎᐅᔾᔨᓂᑦ, ᐅᑯᓐᓂᓂᑦ
ᑲᑎᖅᖏᓪᓕᓴᓂᕐᕕᑕᐅᔪᓂᒃ ᐊᖏᔪᓂᒃ (ᑕᐃᐱᑦ ᓯᑐᕋᐃᒋᑦ, ᑕᓯᐅᑦᔨᔪᐊᒥ/ᓯᓄᓯᓪᓕᖅᑎᑦ ᐃᒪᓕᓂ
ᖃᓗᓗ Bering ᑕᓗᐅᓕᓂ (ᐅᐊᓪᓚᓂᒋᒥ). ᖃᐅᔨᓴᖅᑕᐅᓯᒪᓂᖅᓂᑦ ᑭᓄᔪᐊ ᐃᑲᔪᖅᓯᓕᑕ
ᐃᓯᒪᒋᔭᐅᓯᒪᔪᒥᒃ ᑭᓄᔪᐊ ᕿᑭᑦᑐᓪᓗᑦ ᖃᓗᓗ ᖃᓄᐅᑐᐃᐃᑦ ᐃᑭᕋᓴᖅᓂᓕᕐᑐᐅᑕᐃᑦ ᐊᐅᖃᖅᖏᓂᒪᓕᑕ
ᑕᓯᐅᑦᔨᔪᐊᒥᐅᓂᒃ/ᓯᓄᓯᓪᓕᖅᑎᐅᓂᒃ ᐊᖏᔪᓂᒃ. ᖃᐅᔨᓴᖅᑕᐅᓯᒪᓂᒃ ᓄᓇᖃᖅᖃᖏᑐᒃᑐᓗᓂᒃ
ᑕᓯᐅᑦᔨᔪᐊᒥᐅᑦ ᑲᑎᖅᖏᓪᓕᓴᕐᓂᑦ ᐊᐅᖃᖅᖏᓂᖅᓄᑦᓂᒪᓕᑕ Bering ᐃᒪᓐᓇᐅᓪᓕᑎᓂᓂᒃ
ᑕᐃᓴᐃᓇᖅᓱᓂ ᑕᐃᐱᑦ ᓯᑐᕋᐃᒋᐊᐅᓂᒃ. ᑭᓇᓗ ᐊᖏᔪᓯᒪᓂᖅ ᓯᕐᓗᖅᓇᐅᓕᐅᖏᓐᓂᔭᐃ
ᓄᐊᔪᓕᓐᓂᐊᓂᓕᖅᓄᓂᒃ ᐅᑭᐅᖅᑕᖏᑐᒃᑐᑦ ᐅᐊᖅᓇᖏᓂᓪᓕᑦ ᒪᓕᐅᔨᓯᒪᓕᑦ ᐊᖏᔪᓯᒪᓕᑦ ᐊᖓᓗᓗ
ᐃᓕᓴᕐᓂᓂᖅᑎᑐᒍᑦ ᐊᐅᖃᖅᖏᓂᖅᓄᓂᒃ ᐊᖏᔪᓂᑦᓂᓕᕐᑐᐅᑕᐃᑦ ᐊᖏᔪᓂᑦ.

INTRODUCTION

The bowhead whale (*Balaena mysticetus*) is the largest of three Arctic species of whale inhabiting Canadian waters. Its very size, including a blubber layer which can measure 43 to 50 cm (Montague 1993), made the bowhead a primary target of the European whaling industry in the 18th, 19th and early 20th centuries (Reeves *et al.* 1983, Ross 1993). This intensive and unmanaged commercial hunting resulted in a reduction of numbers of bowhead from a minimum stock size of 452 in Hudson Bay to approximately 100 animals, and from a minimum of 11,759 animals in Davis Strait to approximately 1000 (Woodby & Botkin 1993). These numbers are indicative of the numbers of animals remaining in the stock at the end of the peak harvest decade. As commercial hunting did continue for many years until collapse, the numbers of animals were reduced even further from these estimates of residual stock size.

Since the collapse of the commercial hunt, bowhead have been protected and only a limited aboriginal subsistence hunt allowed to occur in the western Arctic (Reeves 1991). Despite this protection, it is not clear if the eastern Canadian stocks of bowhead are recovering (Finley 1990). Some information suggests that the number of animals inhabiting the eastern Canadian Arctic is growing (Hay, 1997), but recovery rates are uncertain and have generally been considered to be slow (Davis & Koski 1980). Slow recovery of bowhead populations may be due to continued low-level hunting, instability of ice conditions and predation by killer whales (Mitchell & Reeves 1982, Finley 1990). Also, recovery is difficult to monitor and only a substantial change in numbers would be noticeable. Currently, bowhead are listed as "Endangered" by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) (Campbell 1998).

Because of these questions about the status of the eastern Canadian Arctic bowhead and the renewal of subsistence harvests of these animals, there is a need to gather more information about bowhead stocks. Demographic information such as population size, growth and age structure, social structure, life history variation, habitat use, environmental fluctuations and local extinction and colonizations is invaluable to the management and conservation of wildlife (Lande 1988). There are many approaches available to gather such information and when used together, substantial progress may be made in understanding the population being studied. One such tool is the examination of genetic markers. Different classes of markers exist and they are distinct in the type of information that they produce (Milligan *et al.* 1994). Mitochondrial DNA (mtDNA) sequencing is an excellent method for the study of population structure and divergence (Parker *et al.* 1998). Nuclear DNA (nDNA) microsatellite variation can be used to study the level of diversity within and among populations (Parker *et al.* 1998), identification of individuals (Haig 1998) and when applied over a series of unlinked loci, can even test whether population size has been constant or increasing (Goldstein *et al.* 1999). These types of markers have been applied very effectively to investigate questions of population structure and dynamics for a number of cetacean species (e.g. Baker *et al.* 1998; Brown Gladden *et al.* 1997, 1999; Richard *et al.* 1996). Not much work has been done on bowhead, and the genetic studies so far have focused mainly on the Bering/Chukchi/Beaufort Sea and Okhotsk Sea animals (Rooney *et al.* 1999; LeDuc *et al.* 1998). However, these studies did demonstrate that DNA analyses are able to reveal a level of variability useful for the examination of bowhead stock structure.

In this study, we present preliminary analyses of mtDNA and nDNA data for bowhead samples collected in the eastern Canadian Arctic. The main issue addressed

is whether the Hudson Bay/Foxe Basin bowhead are in fact genetically distinct from the Davis Strait animals. We also examine the genetic variation among these two groups and a sample of bowhead from the Beaufort Sea. This information may then clarify the stock designations of bowhead in the eastern Canadian Arctic, which are currently based on inferences from information on commercial catches and geographical barriers such as land masses and ice cover (Moore & Reeves 1993).

MATERIALS AND METHODS

Sample collection

Biopsy samples of bowhead skin were obtained during post mortem examinations of beached and hunted animals and during biopsy sample programs targeting free-ranging whales. The majority of samples were obtained during biopsy sampling programs of free-ranging bowhead whales in Foxe Basin (Igloolik), Repulse Bay, Cumberland Sound (Pangnirtung) and Pond Inlet. Samples from Foxe Basin and Cumberland Sound were collected from June through August, while those in Repulse Bay were collected in August and September. Based on the assumption that northern Hudson Bay bowhead might be distinct from Foxe Basin whales, we avoided collection of samples from Repulse Bay earlier than August to preclude the possibility that whales sampled in Repulse Bay were actually Foxe Basin whales migrating through Repulse Bay.

All biopsy sampling was conducted from a two-person kayak, boat, or from an ice platform. The majority of whales sampled were initially approached by boat and either pursued and fired at from the boat, or alternatively, a kayak was launched from the floe edge or boat and used to approach the whales to within firing range of the biopsy system. Sampling from the floe edge was conducted opportunistically when bowhead were moving along or moving toward and diving beneath the floe edge.

Biopsy tips were cleaned and sterilized using a two stage process involving immersion and cleaning in hydrogen peroxide to dissolve and remove previous genetic material, and then in Betadyne antiseptic solution. Skin samples were transferred from the biopsy tip into vials containing a salt-saturated 20% dimethylsulfoxide (DMSO) solution (Seutin *et al.* 1991) within 1 to 15 minutes of extraction from the whale. These samples were then kept cool until genetic analyses were initiated.

Using all collection methods, a total of 86 biopsy samples were obtained, of which 81 were from free-ranging bowhead. Good representation was obtained for Foxe Basin (Igloolik) (n = 36) and Cumberland Sound (Pangnirtung) (n = 26), followed by substantially less representation for Repulse Bay (n = 13), Hudson Strait (Cape Dorset) (n=1) and Pond Inlet (n = 0). A total of n=9 samples was obtained from free-ranging bowhead in the Mackenzie Delta area (Shingle Point and King Point) in 1990. An additional sample was collected after a bowhead hunt in Shingle Point in 1996, bringing the sample total for this area to n=10.

The difference in sample sizes between regions is generally reflective of a combination of total search effort and number of whales observed. In Pond Inlet, although there were reports of a whale sighting during the sample collection period, no whales were observed by samplers in this region.

DNA analyses

Total cellular DNA was extracted from bowhead skin using the methods described in Maiers *et al.* (1996) with some modifications. The bowhead skin has a very tough, rubbery texture after preservation and it required several weeks of incubation at 37°C and repeated additions of proteinase K (20 mg/mL) to digest the tissue to the point where it was suitable for extraction. Once this process was complete, in most samples sufficient quantities of DNA was recovered for analyses.

A molecular method (Bérubé & Palsbøll 1996) was used to determine the sex of each animal sampled. This information has not been incorporated into the statistical analyses for this document, but will be considered in future analyses of the data.

A portion of the mitochondrial DNA (mtDNA) d-loop was amplified using primers from Arnason *et al.* (1993) and using polymerase chain reaction (PCR) conditions described by Kocher *et al.* (1989). Thirteen individual bowhead samples (n=5 from Mackenzie Delta, n=8 from Igloolik) were initially amplified and sequenced across 900bp of sequence using primer sequences designed from a published bowhead mtDNA sequence (Arnason *et al.* 1993). Sequencing was performed using an ABI Prism 377 automated DNA sequencer and the dRhodamine fluorescent dye terminator chemistry. The resulting sequences were aligned and variable nucleotide positions assessed using MacVector (ver. 3.5, IBI). The region of greatest variability was determined and a final sequencing primer designed to target this region in all subsequent bowhead sample sequencing.

Nuclear DNA was analyzed at 13 microsatellite loci using primer pairs developed for other cetacean species (Table 1). Amplification of the microsatellites was carried out as described in Buchanan *et al.* (1996) with changes to the annealing temperatures determined by which primer pair was being used (Table 1). The amplification products were visualized and the alleles sized using a visual comparison to a standard M13 sequencing reaction or a set of control samples (samples of known sizes that were determined using the sequencing ladder).

For both methods, there were a number of samples that failed to amplify consistently across loci. For the purposes of statistical analyses, this was treated as missing data and reduced the sample size at some locations. Unfortunately, the most difficulty was encountered with the Repulse Bay samples. A suitable explanation for this phenomenon has not yet been formulated, and modifications of laboratory methods are ongoing in a effort to yield useful information from these samples.

Statistical analyses

"Sample populations" that were statistically compared for the purposes of this document were: 1. Mackenzie Delta (Shingle Point and King Point samples); 2. Northern Hudson Bay (Repulse Bay samples); 3. Foxe Basin (Igloolik and Cape Dorset samples); and 4. Davis Strait (Pangnirtung samples). Future analyses of the data will hopefully test the relationship of more samples obtained from other locations in northern Hudson Bay with those of Foxe Basin and at this time the Cape Dorset sample will be separated.

A test for agreement with Hardy-Weinberg equilibrium was performed using Fisher's Exact test (Fisher 1935) available in GENEPOP ver. 3.1b (Raymond & Rousset, 1995) for each locus in each population. Depending on the number of alleles present at

the locus, the test is either an exact Hardy-Weinberg test (Louis & Dempster, 1987) or an unbiased estimation of exact Hardy-Weinberg using a Markov chain method described by Guo & Thompson (1992). In both cases the analysis tests the probability of error when rejecting the null hypothesis that the population, at that locus, is in Hardy-Weinberg equilibrium.

Fisher's Exact Test (in GENEPOP (ver. 3.1b)) was also used to test for differences in the distribution of alleles at each locus among the bowhead populations. This method involves the construction of a contingency table for the data at each locus which is then used to compare allele frequencies between pairs of populations. The probability of error when rejecting the null hypothesis that the allelic distribution is identical across populations was determined. Future statistical analyses will also test for significance differences in the distribution of alleles between males and females.

An Analysis of Molecular Variance (AMOVA) as described by Excoffier *et al.* (1992) and Michalakis & Excoffier (1996) was performed using methods available in Arlequin (ver. 1.1) (S. Schneider *et al.*; http://anthropologie.unige.ch/arlequin). Data from both mtDNA sequencing analyses and nDNA microsatellite analyses may be tested separately or combined. AMOVA compares the distribution of alleles at all loci within and among sample groups, and tests whether or not the observed differentiation is due to chance. Also, AMOVA calculates genetic distances (Fst values) between pairs of sample populations. The Fst value is a measure of the relative value of between population variation and within population variation (with variation measured as the number of alleles differing among individuals within and between populations). This amounts to a weighted Fst statistic over all loci (Weir & Cockerham 1984). The significance of the pairwise Fst values are tested using a non-parametric permutation approach, this determining the probability of the observed or a lower Fst value being due to chance. Again, for these analyses, the bowhead samples were grouped according to sampling location and each group tested as a "sample population". Future analyses will include testing of samples without any pre-determined structure.

Finally, our ability to discriminate individuals from different populations was tested using an "Allocation Test" performed using a Visual Basic program written by B.G.E. de March at the Freshwater Institute. In this approach, every individual was "allocated" to a population by calculating the probabilities of its genotype in each population and then identifying the population in which this probability was largest. These probabilities were based on overall gene frequencies in different populations. The frequency distribution of the allocations was then tabulated. This test was performed in two ways - with and without replacement of the individual back into its source population before the probability was calculated.

RESULTS AND DISCUSSION

Mitochondrial DNA variation

Three-hundred and forty-three base pairs (343bp) of sequence of the mtDNA d-loop was compared in 64 bowhead from four locations (Table 2). Unfortunately, this only represents information from 74% of our available samples, with the largest loss in the Repulse Bay samples (results from only 2 out of 13 samples). This deficit comes from a failure of the extracted DNA to amplify during PCR, which may be due to poor quality DNA recovered during extraction or some type of contamination in the sample that is

carried through to the DNA and interferes with amplification success. Hopefully, these issues will be resolved in the near future and these samples can be processed and their results added to the analyses.

Twenty-four variable nucleotide positions were found in the usable sequences which resulted in the assignment of 22 different haplotypes (Table 3). All of the variability was due to substitution events, of which there were 22 transitions and 2 transversions. Both transversions occurred in haplotypes that were found only in the Pangnirtung sample and both haplotypes were unique to one individual each.

The distribution of haplotypes is quite different among Mackenzie Delta, Igloolik and Pangnirtung samples (Table 2 and Figure 2). The most common haplotype, haplotype 1, made up the highest proportion in both the Mackenzie Delta (3/9 animals) and Igloolik (13/34 animals) samples, but it was not found in the Pangnirtung animals. There was only one haplotype that was found among animals at all three locations (haplotype 5) and this occurred in one animal from each group. The Mackenzie Delta animals overlapped with the Igloolik animals with 2 shared haplotypes and the Igloolik and Pangnirtung animals shared 5 haplotypes. Other than the previously mentioned haplotype 5, Pangnirtung and Mackenzie Delta samples did not share any haplotypes. In all three instances, there were haplotypes unique to the location. The 2 haplotypes generated for Repulse Bay can not really be considered informative for the location, especially since both haplotypes are also found in the Igloolik samples. Thus, there is no basis for a comparison between north Hudson Bay and Foxe Basin.

Significant differences among haplotype frequencies were revealed among all three of the locations ("haplotype" column, Table 5). These results support the hypothesis of three distinct stocks summering in Canadian waters - Bering Sea stock, Hudson Bay stock and Davis Strait stock (Moore & Reeves 1993) (Figure 1). Though initial interpretation of the haplotype distribution appeared to indicate a broader relationship between the western (Mackenzie Delta) and eastern (Igloolik and Pangnirtung) samples, the GENEPOP results provide more details. The significance of the differences between Pangnirtung and Igloolik and Pangnirtung and Mackenzie Delta were greater ($p < 0.005$) than the difference between Igloolik and Mackenzie Delta ($p < 0.05$). This suggests closer relationship between Igloolik and Mackenzie bowhead than between Igloolik and Pangnirtung animals. This idea is further supported by the results of the AMOVA test using genetic distances generated by mtDNA haplotype data (Table 6). These results reveal differences only between Pangnirtung and Igloolik and Pangnirtung and Mackenzie Delta. The Igloolik and Mackenzie Delta comparison did not reject the null hypothesis (no difference between populations).

The results from any analyses involving the Repulse Bay sample are very misleading as the small sample size (n=2) would not be representative of the bowheads summering at that location.

Nuclear DNA variation

Microsatellite analyses at the 13 loci revealed between 2 and 16 different alleles per locus with an average of 6.2 alleles/locus (Table 1). Amplification success was better than for mtDNA analyses, however not all samples produced readable results at all loci. Where the alleles could not be sized with confidence, they were treated as missing data for that sample at that locus. Again, most difficulty occurred with the Repulse Bay samples. One of the 13 samples was discarded from the analyses due to

failure to amplify at almost all loci. Four of the remaining 12 samples had between 4 and 16 of the 13 loci data missing. In such cases AMOVA and Fisher's Exact test compare available data only, hence results may be biased depending on how informative the remaining available data are.

Animals were also compared to determine if any animals in the sample pool had an identical match of alleles at all loci. This would suggest a re-sampling of the same individual either later during the same sampling period or of an animal that had returned to the same location to be sampled in subsequent years. No matches were found, thus all samples were considered to be unique individuals. This technique does, however, have the potential to be a useful way of identifying and/or tracking animals with a "genetic tag" (Palsbøll et al. 1997).

Allele frequencies for Mackenzie Delta and Pangnirtung were not in Hardy-Weinberg Equilibrium (HWE) at 2 loci and for Igloolik at 3 loci ($P < 0.05$) (Table 4). These deviations did not occur consistently at one locus, thus it is unlikely that these results are due to the presence of null alleles (alleles that are not detected within the resolution limits of the laboratory techniques or alleles that are not produced due to a mutation that affects their ability to be detected at all). A possible explanation may be the sampling technique. Several whales from single groups were often biopsied at one time. These animals may be related, thus the genotypic information may skew the calculation of the HWE. Also, a small fraction of samples are expected to not be in HWE due to chance. However, overall results (40 out of 51 tests) support a high degree of agreement with Hardy-Weinberg Equilibrium.

Analyses of genic differentiation at each microsatellite locus between pairs of populations revealed some differences, though the results were not consistent (Table 5). Locus FCB 1 revealed significant differences among all pairs of comparisons. However, none of the other 12 nuclear loci revealed a consistent pattern of differentiation among the populations. Overall, though the results of this analysis indicate that there is some level of variability in the distribution of alleles, no clear distinction of stocks was possible using these data alone.

Results of the AMOVA calculations using the nuclear DNA loci data support the patterns revealed by the mtDNA haplotype analyses. A significant difference was found only in the comparison of animals from the Igloolik and Pangnirtung populations (Table 7). This strengthens the evidence for distinction of genetically separate Hudson Bay/Foxe Basin and Davis Strait stocks. Furthermore, these results indicate that interbreeding of animals from these two stocks may be limited. Conversely, the lack of genetic differentiation between eastern and western Canadian Arctic bowhead may also imply that animals are mixing at some point during their migrations and/or their long life history and perhaps are interbreeding. Analyses of samples obtained from more northern locations within the bowhead distribution (Figure 1) would aid in resolving this issue.

Overall, the ability of the data to discriminate among populations was quite good. When animals were removed from their source population and then "allocated" back to one of the populations (after it was replaced), the individual was correctly allocated over 90% of the time (Figure 3). One out of 37 Igloolik animals was allocated to the Mackenzie Delta and 2 out of 37 were put in the Pangnirtung population. When Pangnirtung was the source population, 2 out of 26 individuals were allocated to Igloolik.

However, many of the individuals may be allocated to their correct sample populations because they have rare or unique alleles, and this may be entirely due to small sample sizes. The allocation test with the individual removed from its source population prior to the population probabilities being calculated may be more appropriate (Figure 4). With this test, a significant number of individuals were not allocated to any source population. These individuals that were "not placed" had genotypes that were not possible at any other location i.e. they had unique genotypes. In all sample locations tested, especially Repulse Bay, the number of samples is obviously not sufficient to represent the total variety of genotypes present in that population.

CONCLUSIONS

The combined results of mitochondrial and nuclear DNA genetic marker analyses support the separation of a discrete Hudson Bay/Foxe Basin stock from the Davis Strait stock. This would suggest that mixing of bowhead during migrations is not occurring, is very limited, or does not influence the accumulation of genetic distinctiveness. However, the relationship of the eastern Canadian Arctic bowhead stocks to the Bering Sea stock may not be as clear. Results in this study indicate that the animals of the Hudson Bay stock are more similar to the Bering Sea stock than the Davis Strait stock. And again, the Davis Strait stock is quite distinct from the Bering Sea stock. The most likely explanation for this is that the Davis Strait stock was colonized after glacial retreat by a separate and genetically distinct group of bowhead from those that colonized the Bering Sea and Hudson Bay. Reeves at al. (1983) recognized that there is a very close association between bowhead distribution and movements and sea ice conditions. This association has been examined historically using information from radiocarbon dating of bowhead bones linked to patterns of driftwood dispersal by sea ice (Dyke & Morris 1990, Dyke *et al.* 1996). These studies revealed that expansions and contractions of ice-covered areas created boundaries to bowhead movements that persisted for millennia (Dyke *et al.* 1996). It was also suggested that during 11000 - 8500 BP a large bowhead population extended in the summer from Beaufort Sea to Baffin Bay, though access to Baffin Bay likely was still blocked by ice (Dyke & Morris 1990). These conclusions support a historical foundation of bowhead distribution patterns that would lead to the population structure revealed by the genetic data in this study.

ACKNOWLEDGEMENTS

The authors would like to thank Brad Parker and the Repulse Bay HTO for the collection of some of the samples used in this study. Logistic support was provided by the communities of Igloolik, Repulse Bay, Pangnirtung and Pond Inlet during the sampling of free-ranging animals. Thank-you to Margaret Friesen, Denise Tenkula and Rob Bajno for providing laboratory assistance. A special thank-you to Elena Valsecchi and Per Palsbøll for the provision of microsatellite primers prior to publication. Funding for this project has been provided by the Department of Fisheries and Oceans Canada, in the Eastern Arctic by the Nunavut Implementation Fund and in the Western Arctic by the Fisheries Joint Management Committee (FJMC).

REFERENCES

Árnason, Ú., A. Gullberg and B. Widegren. 1993. Cetacean mitochondrial DNA control region: sequences of all extant baleen whales and two sperm whale species. Molecular Biology and Evolution 10: 960-970.

Baker, C.S., L. Flórez-González, B. Abernethy, H.C. Rosenbaum, R.W. Slade, J. Capella and J.L. Bannister. 1998. Mitochondrial DNA variation and maternal geneflow among humpback whales of the southern hemisphere. Marine Mammal Science 14: 721-737.

Bérubé, M. and P. Palsbøll. 1996. Identification of sex in cetaceans by multiplexing with three ZFX and ZFY specific primers. Molecular Ecology 5: 283-287.

Brown Gladden, J.G., M.M. Ferguson and J.W. Clayton. 1997. Matriarchal genetic population structure of North American beluga whales *Delphinapterus leucas* (Cetacea: Monodontidae). Molecular Ecology 6: 1033-1046.

Brown Gladden, J.G., M.M. Ferguson, M.K. Friesen and J.W. Clayton. 1999. Population structure of North American beluga whales (*Delphinapterus leucas*) based on nuclear DNA microsatellite variation and contrasted with the population structure revealed by mitochondrial DNA variation. Molecular Ecology 8: 347-363.

Buchanan F.C., M.K. Friesen, R.P. Littlejohn and J.W. Clayton. 1996. Microsatellites from the beluga whale (*Delphinapterus leucas*). Molecular Ecology 5: 571-575.

Campbell, R.R. *Editor*. 1998. Rare and endangered fishes and marine mammals of Canada: COSEWIC Fish and Marine Mammal Subcommittee Status Reports XII. Canadian Field-Naturalist 112: 94-97.

Davis, R.A. and W.R. Koski. 1980. Recent observations of the bowhead whale in the eastern Canadian high Arctic. Report of the International Whaling Commission 30: 439-444.

Dyke, A.S. and T.F. Morris. 1990. Postglacial history of the bowhead whale and of driftwood penetration; implications for paleoclimate, central Canadian Arctic. Geological Survey of Canada Paper 89-24, Canadian Government Publishing Centre, Supply and Services Canada, Ottawa, Canada.

Dyke, A.S., J. Hooper and J.M. Savelle. 1996. A history of sea ice in the Canadian Arctic archipelago based on postglacial remains of the bowhead whale (*Balaena mysticetus*). Arctic 49: 235-255.

Excoffier, L., P. Smouse and J. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131: 479-491.

Finley, K.J. 1990. Isabella Bay, Baffin Island: an important historical and present-day concentration area for the endangered bowhead whale (*Balaena mysticetus*) of the eastern Canadian Arctic. Arctic 43: 137-152.

Fisher, R.A. 1935. The logic of inductive inference. Journal of the Royal Statistics Society 98: 39-54.

Goldstein, D.B., G.W. Roemer, D.A. Smith, D.E. Reich, A. Bergman and R.K. Wayne. 1999. The use of microsatellite variation to infer population structure and demographic history in a natural model system. Genetics 151: 797-801.

Guo, S.W. and E.A. Thompson. 1992. Performing exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48: 361-371.

Haig, S. 1998. Molecular contributions to conservation. Ecology 79: 413-425.

Hay, K. 1997. Inuit bowhead knowledge study interim report: Northwest Hudson Bay and High Arctic. Nunavut Wildlife Management Board, Iqaluit, NT.

Kocher, T.D., W.K. Thomas, A. Meyer, S.V. Edwards, S. Paabo, F.X. Villablanca and A.C. Wilson. 1989. Dynamics of mitochondrial evolution in animals: amplification and sequencing with conserved primers. Proceedings of the National Academy of Sciences of the USA 86: 6196-6200.

Lande, R. 1988. Genetics and demography in biological conservation. Science 241: 1455-1460.

LeDuc, R.G., A. Rosenberg, A.E. Dizon, A.M. Burdin, S.A. Blokhin and R.L. Brownell Jr. 1998. Preliminary genetic analyses (mtDNA and microsatellites) of two populations of bowhead whales. *Working paper SC/50/AS11 presented to the scientific working group at the International Whaling Commission meeting, Oman.*

Louis, E.J. and E.R. Dempster. 1987. An exact test for Hardy-Weinberg and multiple alleles. Biometrics 43: 805-811.

Maiers, L.D., M.K. Friesen, A.V. Wiens and J.W. Clayton. 1996. Use of DNA microsatellites in beluga whale (*Delphinapterus leucas*) population genetics. Canadian Technical Report of Fisheries and Aquatic Sciences 2115, v + 17pp.

Michalakis, Y. and L. Excoffier. 1996. A generic estimation of population subdivision using genetic distances between alleles with special reference for microsatellite data. Genetics 142: 1061-1064.

Milligan, B.G., J. Leebens-Mack and A.E. Strand. 1994. Conservation genetics: beyond the maintenance of marker diversity. Molecular Ecology 3: 423-435.

Mitchell, E.D. and R.R. Reeves. 1982. Factors affecting abundance of bowhead whales *Balaena mysticetus* in the eastern Arctic of North America, 1915-1980. Biological Conservation 22: 59-78.

Montague, J.J. 1993. Introduction, pp. 1-21 in *The Bowhead Whale*, edited by J.J. Burns, J.J. Montague and C.J. Cowles. Allen Press Inc., Lawrence, KS.

Moore, S. and R.R. Reeves. 1993. Distribution and movement, pp. 313-386 in *The Bowhead Whale*, edited by J.J. Burns, J.J. Montague and C.J. Cowles. Allen Press Inc., Lawrence, KS.

Palsbøll, P., M. Bérubé, A.H. Larsen and H. Jørgensen. 1997. Primers for the amplification of tri- and tetramer microsatellite loci in baleen whales. Molecular Ecology 6: 893-895.

Palsbøll, P., J. Allen, M. Bérubé, P.J. Clapham, T.P. Feddersen, P.S. Hammond, R.R. Hudson, H. Jørgensen, S. Katona, A. Holm Larsen, F. Larsen, J. Lien, D.K. Mattila, J. Sigurjónsson, R. Sears, T. Smith, R. Sponer, P. Stevick & N. Ølen. 1997. Genetic tagging of humpback whales. Nature 388: 767-769.

Parker, P.G., A.A. Snow, M.D. Schug, G.C. Booton and P.A. Fuerst. 1998. What molecules can tell us about populations: choosing and using a molecular marker. Ecology 79: 361-382.

Raymond M. and F. Rousset. 1995. GENEPOP (version 3.1b): population genetics software for exact tests and ecumenicism. Journal of Heredity 86: 248-249.

Reeves, R., E. Mitchell, A. Mansfield and M. McLaughlin. 1983. Distribution and migration of the bowhead whale, *Balaena mysticetus*, in the eastern North American Arctic. Arctic 36: 5-84.

Reeves, R.R. 1991. The bowhead whale. *Underwater World*, Communications Directorate, Department of Fisheries and Oceans, Ottawa, Canada.

Richard, K.R., M.C. Dillon, H. Whitehead and J.M. Wright. 1996. Patterns of kinship in groups of free-living sperm whales (Physeter macrocephalus) revealed by multiple molecular genetic analyses. Proceedings of the National Academy of Sciences U.S.A. 93: 8792-8795.

Rooney, A.P., R.L. Honeycutt, S.K. Davis and J.N. Derr. 1999. Evaluating a putative bottleneck in a population of bowhead whales from patterns of microsatellite diversity and genetic disequilibria. Journal of Molecular Evolution, in press.

Ross, W.G. 1993. Commercial whaling in the North Atlantic sector, pp. 511-561 in *The Bowhead Whale*, edited by J.J. Burns, J.J. Montague and C.J. Cowles. Allen Press Inc., Lawrence, KS.

Seutin G., B.N. White and P.T. Boag. 1991. Preservation of avian blood and tissue samples for DNA analyses. Canadian Journal of Zoology 69: 82-90.

Valsecchi, E. and W. Amos. 1996. Microsatellite markers for the study of cetacean populations. Molecular Ecology 5: 151-156.

Weir, B.S. and C.C. Cockerham. 1984. Estimating F-statistics for the analysis of population structure. Evolution 38:1358-1370.

Woodby, D.A. and D.B. Botkin. 1993. Stock sizes prior to commercial whaling, pp. 387-407 in *The Bowhead Whale*, edited by J.J. Burns, J.J. Montague and C.J. Cowles. Allen Press Inc., Lawrence, KS.

Zeh, J.E., C.W. Clark, J.C. George, D. Withrow, G.M. Carroll and W.R. Koski. 1993. Current population size and dynamics, pp. 409-489 in *The Bowhead Whale*, edited by J.J. Burns, J.J. Montague and C.J. Cowles. Allen Press Inc., Lawrence, KS.

Figure 1. Distribution and summer concentrations of bowhead whales in Canadian waters.

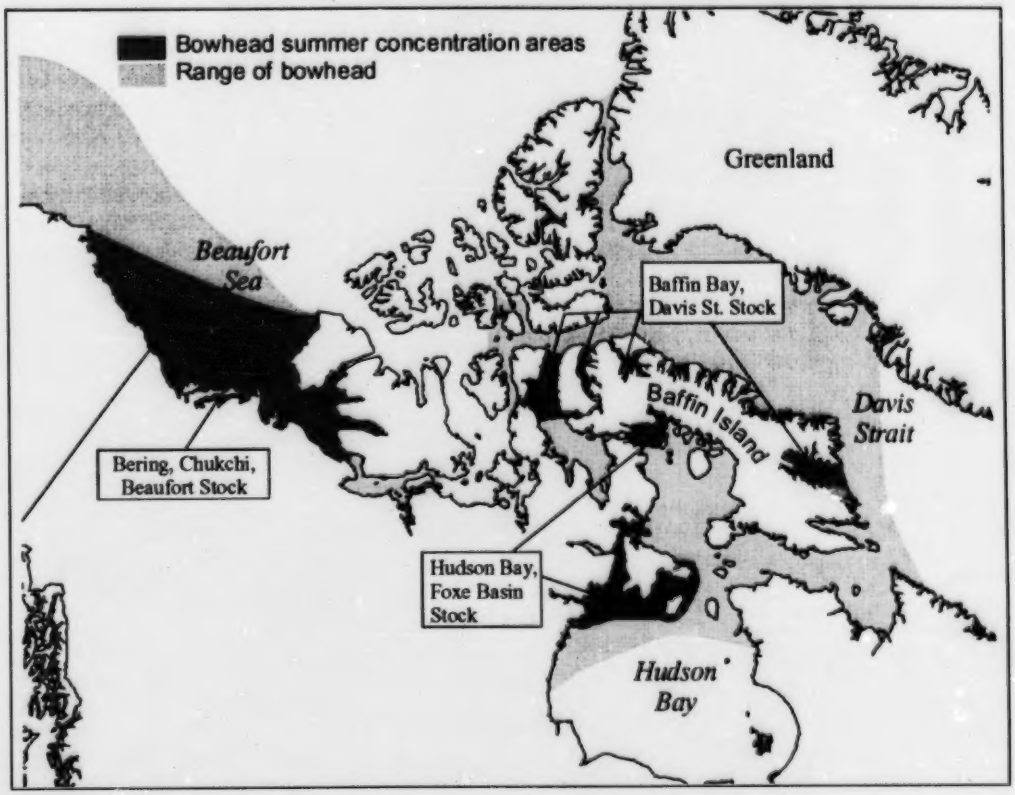


Figure 2 Distribution of haplotypes among bowhead sampled at Pangnirtung, Repulse Bay, Igloolik and the Mackenzie Delta.

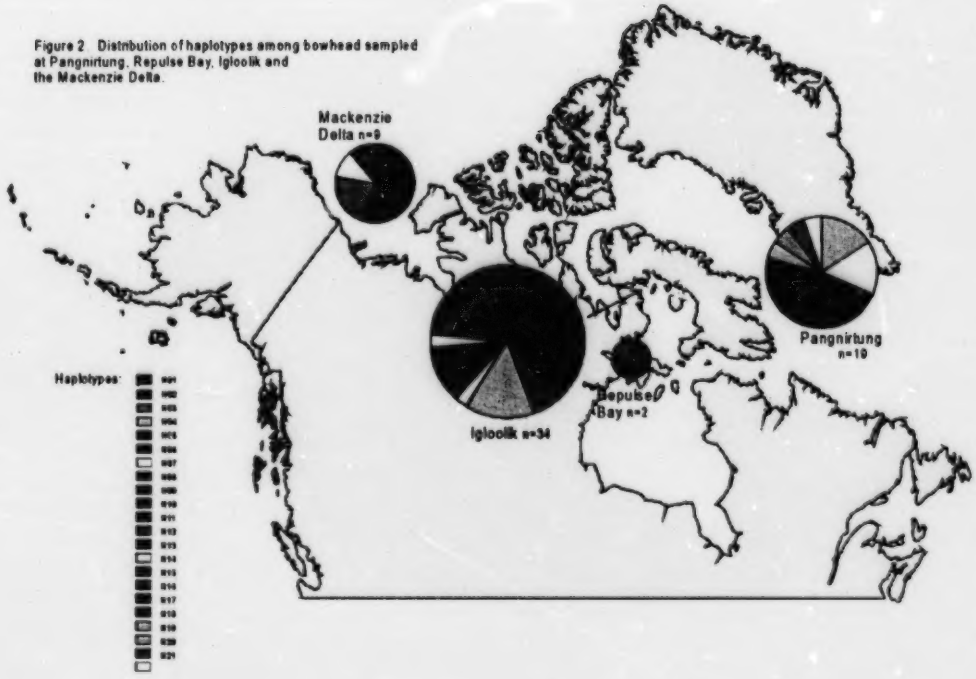
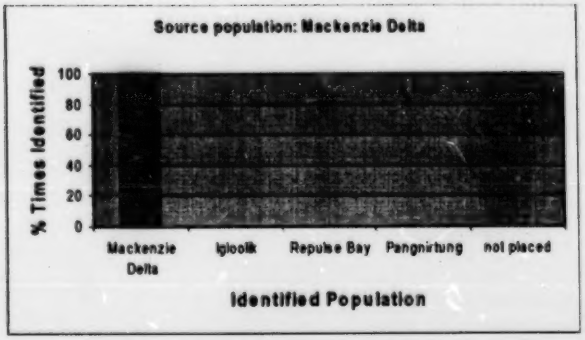
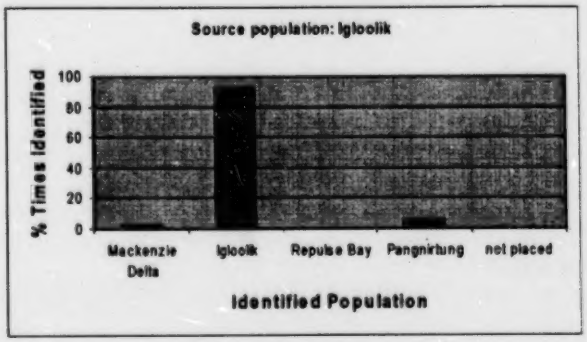
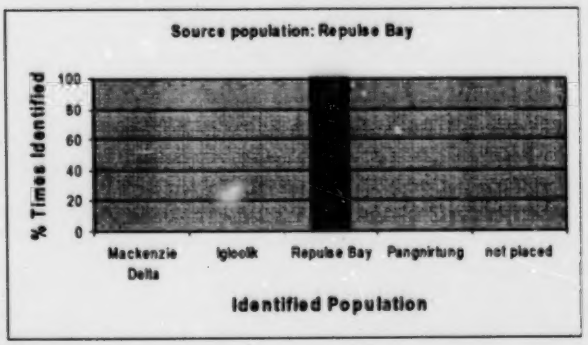


Figure 3. Results of "Allocation" method used to test ability of genetic data for discriminating populations. In this test, a test individual was removed from a source population and then replaced. The probability was then calculated that this individual was a member of each population. It was then allocated to the population where its expected genotype was highest. For further explanation, see text.







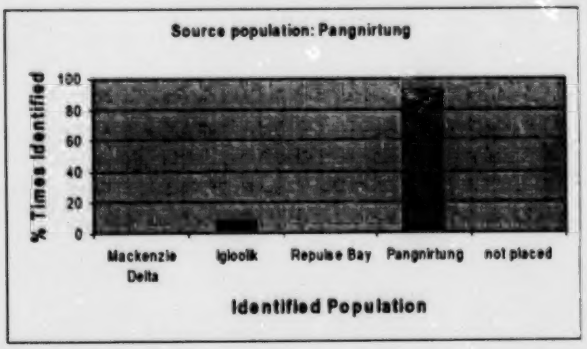
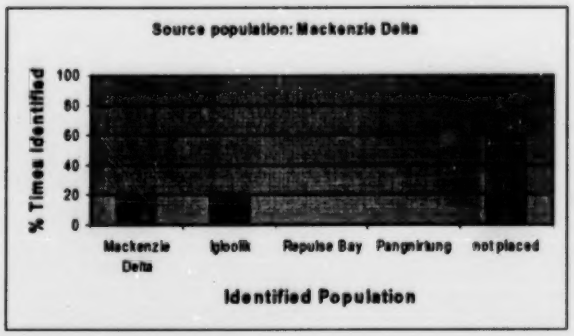
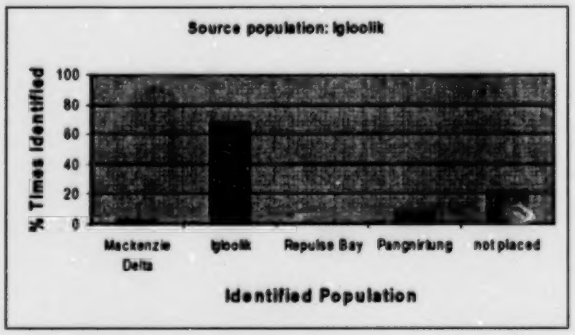
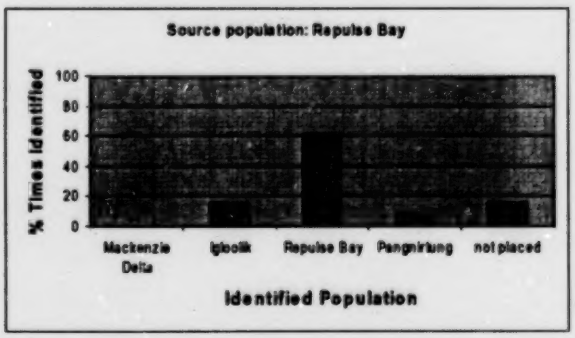


Figure 4. Results of "Allocation" method used to test ability of the genetic data for discriminating the populations. For each population, individuals were removed from the group and the probability was calculated that this individual was a member of each population. It was then allocated to the population where its expected genotype was highest. Individuals that were "not placed" had genotypes that were not possible at other locations. For further explanation, see text.







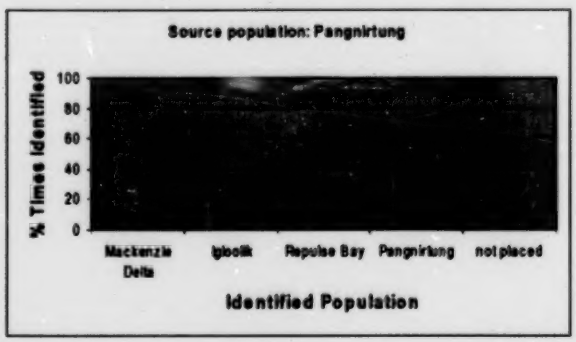


Table 1. Summary of microsatellite primer pairs and annealing temperatures used for nDNA analyses in bowhead.

Microsatellite primer pair	Labelled primer	Annealing temp. (C)	Species derived from	Number of alleles	Reference
DlrFCB 1a and 1b	DlrFCB 1b	64	beluga	4	Buchanan *et al.*(1996)
DlrFCB 4a and 4b	DlrFCB 4b	63	beluga	16	Buchanan *et al.*(1996)
DlrFCB 5a and 5b	DlrFCB 5b	61	beluga	4	Buchanan *et al.*(1996)
DlrFCB7a and 7b	DlrFCB 7b	61	beluga	2	Buchanan *et al.*(1996)
DlrFCB 11a and 11b	DlrFCB 11b	61	beluga	7	Buchanan *et al.*(1996)
DlrFCB 13a and 13b	DlrFCB 13a	61	beluga	4	Buchanan *et al.*(1996)
Dlr FCB 14a and 14b	DlrFCB 14b	61	beluga	4	Buchanan *et al.*(1996)
EV1a and EV1b	EV1b	50/58	sperm whale	7	Valsecchi & Amos (1996)
EV21a and EV21b	EV21b	52/60	sperm whale	3	Valsecchi & Amos (1996)
EV37a and EV37b	EV37b	59/60	humpback	4	Valsecchi & Amos (1996)
EV104a and EV104b	EV104b	50/58	humpback	10	Valsecchi & Amos (1996)
GATA028F and 028R2	028F	54	humpback	10	Palsboll *et al.* (1998)
GATA098F and 098R	098R	54	humpback	6	Palsboll *et al.* (1998)

Table 2. Haplotype distribution summary, by location, for bowhead whale samples analyzed in this study.

Location	Haplotype 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	Total
Mackenzie Delta	3	-	-	-	1	-	-	-	-	-	1	2	-	1	1	-	-	-	-	-	-	-	9
Foxe Basin (Igloolik)	13	2	5	1	1	3	1	3	2	1	-	-	2	-	-	-	-	-	-	-	-	-	34
Repulse Bay	1	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	-	-	2
Pangnirtung	-	-	3	3	1	1	-	-	3	-	-	-	-	-	-	1	2	1	1	1	1	1	19

Table 3. Mitochondrial DNA haplotypes observed among bowhead whale samples analyzed in this study.

	Sequence Position Number																							
**	143	145	207	228	255	256	259	266	267	273	276	285	286	292	321	356	387	391	405	412	414	444	451	468
	7	9	71	92	119	120	123	130	131	137	140	149	150	156	185	218	251	255	269	276	278	308	315	332
	G	A	G	C	G	G	T	T	C	A	T	C	T	C	G	C	T	C	T	G	T	G	C	T
Haplotype																								
1*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	-	-
3	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
4	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-
6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	-	-	T	-
7	A	-	-	-	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-	T	-	-	-	-	-	-	-	-
9	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	A	T	-
10	-	-	-	-	-	-	-	C	-	-	C	-	-	-	-	-	-	-	C	-	-	-	T	-
11	-	-	-	T	-	-	-	-	-	-	-	-	-	T	-	T	-	-	-	-	-	A	-	-
12	A	-	-	-	-	-	-	-	-	-	-	-	-	T	-	-	-	-	-	A	C	A	-	-
13	-	-	-	-	-	-	-	C	-	-	-	T	-	-	-	-	-	-	C	-	-	-	T	C
14	A	G	-	-	A	-	C	-	T	-	-	-	-	-	-	-	C	-	-	-	-	-	-	-
15	A	-	-	T	A	-	C	-	T	-	-	-	C	-	-	-	C	T	-	-	-	-	T	-
16	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-
17	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	-	-	-	T	-
18	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	C	A	-	-
19	-	-	-	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20	A	-	-	-	A	T	-	-	-	-	C	-	-	T	-	-	-	-	-	-	-	A	-	-
21	A	G	-	-	A	-	C	-	T	-	-	-	C	-	-	-	C	-	-	-	-	-	-	-
22	A	-	T	-	A	-	C	-	T	-	-	-	-	T	A	-	-	-	C	-	-	A	-	-

* Same sequence reported by Arnason, Gullberg and Widegren, Mol. Biol. Evol. **10** (1993)
** Sequence positions reported by Arnason et al. (1993)

Table 4. Results of Hardy-Weinberg test for each locus in each group of bowhead samples (GENEPOP ver. 3.1b). The test provides the probability of error when rejecting the null hypothesis that the population is in HW equilibrium. *P*-values < 0.05 are considered to reject the null hypothesis.

	P-value at each locus:												
Location	FCB 1	FCB4	FCB 5	FCB 7	FCB 11	FCB 13	FCB 14	EV 1	EV 21	EV 37	EV 104	PER 28	PER 98
Mackenzie Delta	0.1331	0.9681	0.3412	0.0526	0.1045	**XX****	0.3169	1.0000	**0.0464**	1.0000	0.4569	0.5637	1.0000
Igloolik	**0.0002***	0.2135	**0.0170**	**0.0137**	0.5493	1.0000	0.8019	0.2572	0.4392	0.4826	0.9935	0.0846	0.6988
Repulse Bay	0.4799	0.8157	0.0977	0.7690	**0.0330**	1.0000	0.6380	1.0000	0.4037	0.2145	**0.0269**	**0.0223**	**0.0347**
Pangnirtung	1.0000	0.0635	0.2762	**0.0040**	0.4972	0.1445	1.0000	0.2167	0.2567	0.4117	0.3857	**0.0000**	0.2810

* Samples in bold indicate loci at which samples are not in Hardy-Weinberg equilibrium.
** "XX" indicates loci at which equilibrium could not be calculated due to the locus being monomorphic or where 2 alleles occurred, but one was found in only one copy.

Table 5. Genic differentiation among the different bowhead population pairs determined by comparison of allelic distributions at each locus (Fisher's Exact Test calculated in GENEPOP ver. 3.1b).

	P-value (probability of error when rejecting Ho*) for each locus													
Population pair	FCB 1	FCB 4	FCB 5	FCB 7	FCB 11	FCB 13	FCB 14	EV 1	EV 21	EV 37	EV 104	GATA028	GATA098	haplotype
Mackenzie & Igloolik	**0.0358****	**0.0171**	1.0000	0.2008	0.5239	0.4935	0.5336	0.2066	0.2523	0.8654	0.7855	0.7825	0.4733	**0.0311**
Mackenzie & Repulse Bay	**0.0009**	0.3098	0.0536	1.0000	0.3673	1.0000	0.5914	**0.0104**	0.5882	0.4493	0.8039	**0.0000**	0.8848	0.7400
Mackenzie & Pangnirtung	**0.0014**	0.1879	0.2724	0.7120	0.3336	0.3518	0.5176	0.5401	0.1485	0.9233	0.0739	0.0829	0.5660	**0.0042**
Igloolik & Repulse Bay	**0.0000**	**0.0053**	0.1734	0.1173	**0.0280**	0.2375	0.1548	0.2074	0.4304	0.5406	0.2676	**0.0000**	0.2778	0.7639
Igloolik & Pangnirtung	**0.0059**	0.3287	0.2630	**0.0143**	0.7466	0.5468	0.8222	0.6826	0.2647	0.4636	**0.0220**	0.1532	**0.0450**	**0.0010**
Repulse Bay & Pangnirtung	**0.0332**	**0.0048**	0.2312	1.0000	0.0599	0.1258	0.1519	0.0911	0.0877	0.4538	**0.0362**	**0.0000**	0.2941	0.7161

* Ho = "The allelic distribution of alleles is identical across populations".
** **Bold** typeface indicates a significant difference in allelic distribution of alleles in the population pair.

Table 6. Analysis of molecular variance (Excoffier et al. 1992, Michalakis and Excoffier 1996) from mtDNA haplotype information. This test (in Arlequin ver. 1.1) calculates and tests pairwise genetic distances between pairs of populations.

	Fst *P*-values*		
	Mackenzie Delta	Igloolik	Repulse Bay
Igloolik	0.3686		
Repulse Bay	0.5358	0.7734	
Pangnirtung	**0.0302****	**0.0312**	0.4663

* The null hypothesis is that there is no difference between populations. The *P*-value indicates the proportion of permutations leading to a Fst value larger than the observed one.
** **Bold** typeface indicates significant difference between populations ($P < 0.05$)

Table 7. Analysis of molecular variance (Excoffier et al. 1992, Michalakis and Excoffier 1996) from genotype information of 13 microsatellite loci. This test (in Arlequin ver. 1.1) calculates and tests pairwise genetic distances between pairs of populations.

	Fst *P*-values*		
	Mackenzie Delta	Igloolik	Repulse Bay
Igloolik	0.3666		
Repulse Bay	0.6496	0.0544	
Pangnirtung	0.1098	**0.0363****	0.0665

* The null hypothesis is that there is no difference between populations. The *P*-value indicates the proportion of permutations leading to a Fst value larger than the observed one.
** **Bold** typeface indicates significant difference between populations ($P < 0.05$)